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Role of glycoprotein Ia gene polymorphisms in determining platelet function in myocardial infarction patients undergoing percutaneous coronary intervention on dual antiplatelet treatment

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Abstract

Response variability to antiplatelet treatment has been described and the widespread use of acetylsalicylic acid (ASA) and clopidogrel requires clarification of the residual platelet reactivity (RPR). Various glycoprotein Ia (GpIa) polymorphisms have been investigated, but their influence on platelet reactivity in myocardial infarction (MI) patients undergoing percutaneous coronary intervention (PCI) on dual antiplatelet treatment is not still elucidated.

Aim of this study was to evaluate the effect of C807T, G873A and T837C polymorphisms of GpIa on modulating platelet function in MI patients on dual antiplatelet treatment undergoing PCI.

We measured platelet function by both a point-of-care assay (PFA100) and platelet-rich-plasma aggregation in 289 MI patients undergoing PCI and receiving dual antiplatelet treatment.

Our data show that C807T/G873A polymorphisms, but not T837C, are associated with higher platelet reactivity. Carriers of the 807T/873A allele had significantly higher platelet aggregation values after arachidonic acid (AA) and collagen stimuli and, even if they did not reach the statistical significance, after 2 and 10 μM ADP stimuli; 807T/873A allele carriers had also significantly shorter closure times on PFA100/epinephrine membranes. At the multiple analyses, C807T/G873A polymorphisms resulted an independent risk factor for RPR defined by both AA induced platelet aggregation (OR = 3.0, 95%CI 1.17–7.89, $p = 0.022$) or by PFA100/epinephrine (OR = 4.1, 95%CI 1.53–10.89, $p = 0.005$).

In conclusion, this study shows the 807T/873A allele of the GpIa gene is an independent risk factor for the RPR on dual antiplatelet treatment, and extends, in a larger acute coronary syndrome population, the observation that the 807T/873A allele is associated with higher platelet reactivity.

Keywords: Glycoprotein Ia polymorphisms; Residual platelet reactivity; Aspirin resistance; Antiplatelet treatment; Percutaneous coronary intervention

1. Introduction

Acetylsalicylic acid (ASA) and clopidogrel have become standard therapy in patients undergoing percutaneous coronary intervention (PCI); however, there is a broad variability in response of individual patients to dual antiplatelet treatment [1–5]. Previous studies estimated that adequate antiplatelet effects are not achieved in 5–45% of patients taking ASA and 4–30% of patients taking clopidogrel [1,3–6]. Some studies are available in the literature which investigated the clinical implications of antiplatelet drug effect resistance

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in chronic coronary artery disease (CAD) or stent thrombosis [2,5,7–10]. Recently, we have demonstrated that post-PCI residual platelet reactivity (RPR), the so called ASA resistance, is an independent predictor of 1-year major adverse coronary events in patients with acute myocardial infarction (MI) [11].

Collagen is a major adhesion protein exposed to platelets after blood vessel injury [12]. Interaction between collagen and blood platelets results in their adhesion, activation and aggregation, and finally in the formation of haemostatic plugs [13,14]. Platelets interact with collagen via several platelet surface receptors, and major roles are attributed to glycoprotein Ia/IIa (GpIaIIa or $\alpha_2\beta_1$ integrin) [15,16] as well as glycoprotein VI [17]. GpIa/IIa is the major collagen receptor involved in the early steps of platelet adhesion to collagen under high shear stress [14]. Previous studies showed that GpIa/IIa is activated by various agonists, including thrombin or ADP, and that the activation of GpIa/IIa leads to a different affinity to collagen [13,18].

Several polymorphisms have been described in the cDNA coding the GpIa subunit. The C807T, T837C and G873A polymorphisms are conservative and do not alter the deduced amino acid sequence of the translated protein. The two linked silent GpIa dimorphisms, C807T and G873A, were correlated with a variable expression of the platelet surface receptor: the genotype 807TT/873AA was associated with a higher receptor density and the genotype 807CC/873GG with a lower density, whereas heterozygous individuals expressed intermediate receptor levels [19,20]. Differences in the receptor density directly correlate with the rate of platelet adhesion to collagen under flow conditions [21]. The functional role of the C807T GpIa polymorphism on modulating the response to antiplatelet drugs in patients has been hypothesized in two previous studies: one studying 44 patients undergoing PCI during the first 24 h following clopidogrel loading dose and on ASA treatment for at least 7 days [22], and the other one evaluating 82 CAD patients on dual antiplatelet treatment for >1 month [23]. Scarce data are available on T837C GpIa polymorphism.

Therefore, the aim of this study was to assess the role of the C807T, G873A, and T837C GpIa gene polymorphisms on modulating platelet function and determining antiplatelet drug response in patients on dual antiplatelet treatment.

2. Materials and methods

2.1. Study population

The study population included 289 consecutive patients admitted to the Coronary Care Unit of the Azienda Ospedaliero-Universitaria Careggi, University of Florence with diagnosis of acute MI. Acute MI was diagnosed on the basis of an increase in creatine kinase MB isoenzyme at least twice the upper normal limits (3.6 ng/mL), and/or elevated cardiac troponin I (cTnI) (>0.15 ng/mL) levels with at least one of the following: acute onset of prolonged (≥20 min) typical ischemic chest pain; ST-segment elevation of at least 1 mm in two or more contiguous electrocardiographic leads or ST-depression of ≥0.5 mm, 0.08 s after the J point in ≥2 contiguous leads, or T waves inversion >1 mm in leads with predominant R waves. In 175 patients ST segment elevation MI (STEMI) and in 114 non-ST segment elevation MI (NSTEMI) was diagnosed. All patients underwent coronary angiography performed by the Judkins' technique and primary PCI. Patients were considered to have hypertension if they had been diagnosed as hypertensive according to the European Society of Hypertension/European Society of Cardiology guidelines or were taking antihypertensive drugs. Dyslipidemia was defined according to the Third report of the National Cholesterol Education Program and diabetes according to the American Diabetes Association.

The exclusion criteria included history of bleeding diathesis, platelet count ≤100,000/mm^3, hematocrit ≤30%, creatinine ≥4.0 mg/dL, and glycoprotein (Gp) IIb/IIIa inhibitor use.

Informed written consent was obtained from all patients and the study was approved by the local Ethical Review Board.

2.2. Patient characteristics

Demographic and clinical characteristics of patients are reported in Table 1.

All patients received a loading dose of 300 mg clopidogrel p.o. before the procedure and of 500 mg ASA i.v., followed by 75 mg clopidogrel and 100 mg ASA daily. Unfractioned heparin 70 IU/kg was used during procedure as anticoagulant.

2.3. Blood sampling

Venous blood samples were taken from each patient 24 h after PCI intervention in tubes containing 3.2% trisodium citrate.

Table 1
Demographic and clinical characteristics of studied subjects

Age	67.5 (27–89)
Sex (M/F)	222/67
Hypertension	178 (61.6%)
Smoking habit	139 (48.1%)
Dyslipidemia	200 (69.2%)
Diabetes	61 (21.1%)
Drugs	
β-blockers	179 (61.9%)
Ca-antagonists	48 (16.6%)
Statins	250 (86.5%)
ACE-inhibitors	237 (82.0%)

2.4. Assessment of platelet aggregation on platelet-rich plasma

Platelet aggregation was assessed using platelet-rich plasma (PRP) by the turbidimetric method in a four-channel aggregometer (APACT 4, Helena Laboratories Italy). Platelet agonist included 2 μmol/L adenosine 5′ diphosphate (2 μM ADP), 10 μmol/L 10 μM ADP, 2 μg/mL collagen, and 0.5 mg/mL arachidonic acid (AA). PRP was obtained as supernatant after centrifugation of citrated blood at 800 rpm for 10 min. Platelet-poor plasma (PPP) was obtained by a second centrifugation of the blood fraction at 2500 rpm for 10 min. Light transmission was adjusted to 0% with PRP and to 100% with PPP for each measurement. Curves were recorded for 6 min and analyzed according to international standards. According to the Born's method, platelet aggregation was determined as the maximal percent change in light transmittance from baseline using PPP as reference in response to different stimuli (2, 10 μM ADP, AA, and collagen). Platelet count was assessed at all time points to ensure that the degree of platelet function was not biased by the number of platelets. The normal reference laboratory values were: 2 μM ADP = 75.5 (43–92)%; 10 μM ADP = 76 (61–100)%; collagen = 80 (58–100)%; AA = 81.5 (69–100)%.

2.5. Assessment of platelet function by whole blood platelet function analyzer (PFA100)

The PFA100 device (Dade-Behring) was used to measure platelet function at high shear conditions on whole citrate blood according to manufacturer instructions. The method determines the time to occlusion of an aperture in a membrane coated with collagen and adenosine diphosphate (closure time CT/ADP) or epinephrine (closure time CT/EPI).

The normal reference laboratory values were: CT/ADP = 97 (60–151) s; CT/EPI = 142 (89–204) s.

2.6. Antiplatelet response

According to previous publications and to our normal laboratory values, the following definitions were used:

(1) Residual platelet reactivity evaluated by AA induced platelet aggregation (AA-RPR) = AA (0.5 mg/mL)-induced platelet aggregation ≥20% [5].
(2) Residual platelet reactivity evaluated by CT/EPI PFA100 (PFA100/EPI-RPR) = CT/EPI <203 s (95th percentile of control distribution).
(3) Residual platelet reactivity evaluated by ADP induced platelet aggregation (ADP-RPR) = 10 μM ADP-induced platelet aggregation ≥70% (90th percentile of control distribution).

2.7. DNA extraction

Genomic DNA was isolated from whole blood by using the FlexiGene DNA kit (QIAGEN, Germany).

2.8. C807T, G873A and T837C GpIa gene polymorphisms detection by electronic microchip

The three polymorphisms were analyzed by using the NanoChip Molecular Biology Workstation and the NanoChip cartridge (Nanogen). The protocol was extensively described in a previous paper [24]. GpIa gene sequences were obtained from GeneBank (www.ncbi.nlm.nih.gov, accession number NM_002203). The used oligonucleotides for PCR and for hybridization, and the melting, annealing, and stringency temperatures are reported in Table A (supplementary appendixes). The software of the system directly assigned the genotype to each sample.

2.9. Statistical analysis

Statistical analysis was performed using the SPSS statistical package Version 11.5. We tested the allele frequencies conformed to Hardy–Weinberg equilibrium proportions by the Chi-square test. Pairwise linkage disequilibrium (LD) was measured by SNPAlyze software (Dynacom). Haplotype reconstruction analysis was performed by using Phase Version 2.1 Software. Genotype and allele frequencies were compared between groups by Chi-square analysis. Categorical variable are expressed as frequencies and percentages. Unless otherwise indicated, data are given as median values and range. Comparisons of continuous variables between patients with and without RPRs or among genotypes were performed by using the non-parametric Mann–Withney *U*-test or the analysis of variance (Kruskal–Wallis). Multiple logistic regression analysis was used to estimate odds ratios and 95% confidence intervals (CI) of the development of AA-RPR and PFA100/EPI-RPR as a function of C807T/G873A polymorphisms and GpIa haplotypes. All odds ratios were adjusted for age, gender and atherosclerotic risk factors, such as hypertension, diabetes mellitus, hypercholesterolemia, obesity, and smoking. A value of $p < 0.05$ was chosen as the cut-off level for statistical significance.

3. Results

In Table 1 demographic and clinical characteristics of patients are reported.

Patients, with or without RPR evaluated by both AA induced platelet aggregation (AA-RPR) and by PFA100 system (PFA100/EPI-RPR), were analyzed for two in-hospital outcomes, acute intra-stent thrombosis (8 out of 289 patients, 2.8%) and mortality for cardiac causes (2 out of 289 patients, 0.7%). Patients with RPR showed a higher percentage of acute intra-stent thrombosis (5.6% versus 1.8%

for PFA100/EPI-RPR, $p = 0.09$, and 5.1% versus 2.2% for AA-RPR, $p = 0.22$), even if it did not reach the statistical significance. No significant differences for the in-hospital mortality for cardiac causes were observed between patients with or without RPR evaluated by both AA-RPR and by PFA100/EPI-RPR.

C807T, G873A and T837C GpIa gene polymorphisms were in Hardy–Weinberg equilibrium.

The C807T and G873A polymorphisms resulted in complete linkage disequilibrium.

The genotype distribution of the C807T/G873A polymorphisms and T837C polymorphism in the overall PCI patient population are shown in Table 2. The rare allele frequency of C807T/G873A and of T873C polymorphisms was 0.360 and 0.112, respectively.

The genotype distribution and allele frequencies of the C807T/G873A polymorphisms and T837C polymorphism were not different in relation to the two in-hospital outcomes (data not shown).

3.1. GpIa polymorphisms and PRP aggregation

Platelet aggregation after different stimuli varied in the overall patient population according to the C807T/G873A genotypes. Carriers of the 807T/873A allele had higher platelet aggregation values than noncarriers of the 807T/873A allele after stimuli with AA and collagen ($p = 0.016$ and $p = 0.020$, respectively) and, even if they did not reach the statistical significance, after 2 μM ADP and 10 μM ADP stimuli ($p = 0.071$ and 0.067, respectively) (Table 2). In particular, patients with 807TT/873AA genotype had higher platelet aggregation values after AA and collagen stimuli with respect to subjects with 807CT/873GA and 807CC/873GG genotypes (Table 2).

Differences observed in platelet aggregation values after the 4 different stimuli in the overall studied population according to T837C genotypes were not statistically significant (Table 2); however, subjects with 837TT genotype showed higher platelet aggregation values after all the four stimuli (Table 2).

Due to the observation of higher platelet aggregation values in subjects with 807TT/873AA and 837TT genotypes, we analyzed the effect of their genotype combinations. The combination of the polymorphisms did not evidence an interaction in inducing higher platelet aggregation values (Table 3).

3.2. GpIa polymorphisms and whole blood platelet function by PFA100

In the whole group studied, CT/EPI values among the different C807T/G873A genotypes were statistically different [$p = 0.003$; 300 (30–300) 807CC/873GG; 300 (90–300) 807CT/873GA; 300 (93–300) 807TT/873AA]. Even if the median CT/EPI value for 807TT/873AA genotype was 300 s, a higher number of subjects with this genotype had CT/EPI values lower than 203 (13.2% 807CC/873GG, 29.6%

Table 2
Distribution of maximal platelet aggregation after different stimuli in the overall study population according to C807T/G873A and T837C GpIa genotypes

	C807T/G873A GpIa genotypes						T837C GpIa genotypes		
	CC/GG	CT/GA	TT/AA	p (overall)	CT/GA + TT/AA	p (vs. CC/GG)	TT	TC + CC	p
Subjects N (%)	114 (39.4)	142 (49.1)	33 (11.4)		175 (60.6)		227 (78.5)	62 (21.5)	
Aggregation according to stimulus (%)									
Arachidonic Acid (0.5 mg/mL)	10.0 (3–91)	11.5 (4–99)	15.0 (5–85)	0.018	12.0 (4–99)	0.016	11.5 (3–91)	9.0 (3–80)	0.052
Collagen (2 μg/mL)	24.0 (1–98)	29.0 (1-99)	34.0 (3–80)	0.054	30 (1–99)	0.020	27.0 (1–99)	21.0 (1–79)	0.222
ADP (2 μM)	23.0 (1–84)	28.0 (1–80)	25.0 (3–91)	0.168	28.0 (1–91)	0.071	27.0 (1–91)	24.0 (1–84)	0.373
ADP (10 μM)	46.0 (6–94)	52.0 (8–89)	54.0 (5–98)	0.170	52 (5–98)	0.067	52.0 (5–98)	46.0 (6–89)	0.151

Table 3
Maximal platelet aggregation after different stimuli in the overall study population according to C807T/G873A and T837C GpIa genotype combinations

	C807T/G873A GpIa and T837C GpIa genotype combinations		*p*-Value
	807/873 TT/AA 837TT	Others	
Subjects *N* (%)	33 (11.5)	256 (88.5)	
Aggregation according to stimulus (%)			
Arachidonic Acid (0.5 mg/mL)	15.0 (5–85)	11.0 (3–91)	0.052
Collagen (2 μg/mL)	34.0 (5–80)	25.0 (1–99)	0.128
ADP (2 μM)	27.0 (1–84)	25.0 (3–91)	0.736
ADP (10 μM)	52.0 (5–98)	51.0 (6-94)	0.802

807CT/873GA, 39.4% 807TT/873AA). No differences in the CT/EPI among patients carrying various T837C polymorphism were detected [$p = 0.53$; 300 (77–300) 837TT; 300 (30–300) 837TC + 837CC].

No differences in the CT/ADP values among patients carrying various C807T/G873A polymorphisms [$p = 0.59$; 102 (7–300) 807CC/873GG; 100 (56–300) 807CT/873GA; 98 (61–300) 807TT/873AA], or T837C polymorphism [$p = 0.51$; 103 (7–300) 837TT; 98 (58–300) 837TC + 837CC].

3.3. GpIa polymorphisms and antiplatelet drug response

Of 289 enrolled patients, 59 (20.4%) showed AA-RPR. The distribution of the T837C genotypes was similar in patients with and without AA-RPR (Fig. 1, panel B), whereas a significant difference ($p = 0.045$) in genotype distribution of C807T/G873A polymorphisms between patients with and without AA-RPR was observed (Fig. 1, panel A).

Seventy-one patients out of 289 studied patients (24.6%) met the definition of PFA100/EPI-RPR. The distribution of the T837C genotypes was similar in patients with and without PFA100/EPI-RPR (Fig. 2, panel B), whereas a significant difference ($p = 0.001$) in genotype distribution of C807T/G873A polymorphisms between patients with and without PFA100/EPI-RPR was observed (Fig. 2, panel A).

The groups of patients with and without AA-RPR or PFA100/EPI-RPR were homogeneous for demographic and clinical characteristics (data not shown).

Forty-one patients out of 289 patients (14.2%) met the definition of ADP-RPR. Demographic and clinical characteristics between patients with and without ADP-RPR were similar (data not shown). No differences between patients

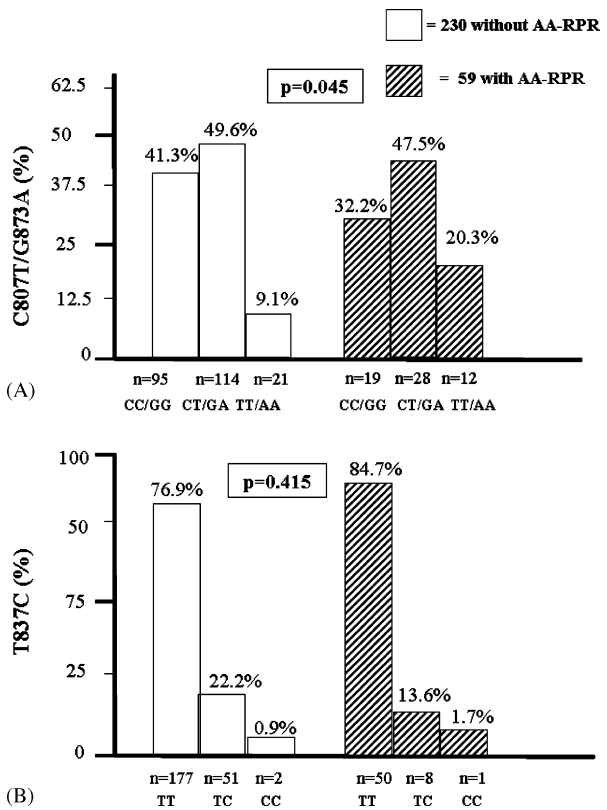

Fig. 1. Genotype distribution of C873T/G873A (panel A) and T837C (panel B) GpIa gene polymorphisms in patients with and without AA-RPR.

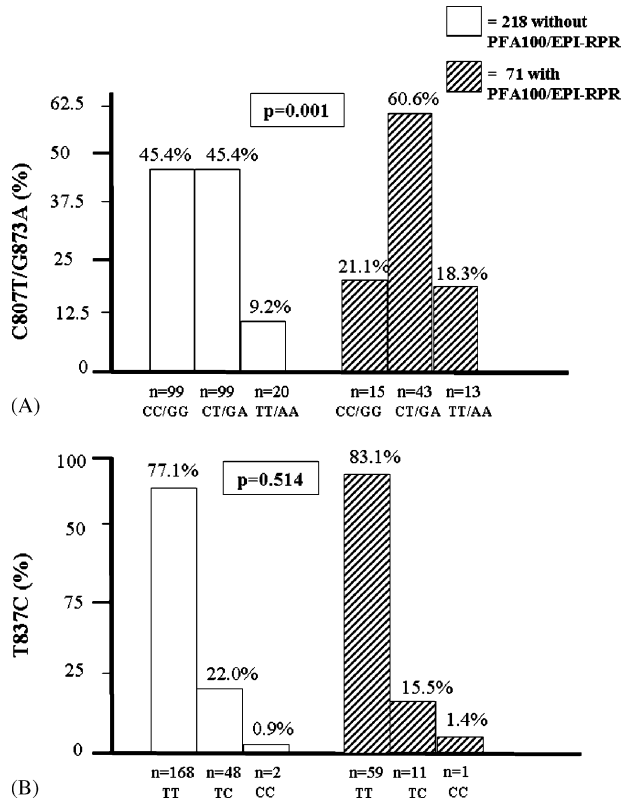

Fig. 2. Genotype distribution of C873T/G873A (panel A) and T837C (panel B) GpIa gene polymorphisms in patients with and without PFA100/EPI-RPR.

Table 4
Haplotype distribution according to AA-RPR and to PFA100/EPI-RPR

Haplotype	AA		PFA	
	With RPR	Without RPR	With RPR	Without RPR
CTG	0.480	0.545	0.434	0.563
TTA	0.435	0.343	0.484	0.323
CCG	0.085	0.112	0.082	0.114

The C807T, T837C, and G873A SNPs in haplotypes are reported from 5′–3′ end of the GpIa gene.

with and without ADP-RPR were observed in C807T/G873A (807TT/873AA 34.1 and 40.3%; 807CT/873GA 58.5 and 47.6%; 807CC/873GG 7.3 and 12.1%, respectively) and T837C (CC + TC 13.9 and 22.6%; TT86.1 and 77.4%, respectively) polymorphism genotype distributions.

In Table 4 the three GpIa polymorphism haplotype distribution according to AA-RPR and to PFA100/EPI-RPR was reported. The GpIa polymorphism haplotype distribution according to PFA100/EPI-RPR was significantly different in patients with and without RPR ($p = 0.0021$), whereas according to AA-RPR showed only a trend ($p = 0.156$). In particular, the allele frequency of the TTA (807T/837T/873A) haplotype was higher in patients with RPR with respect to patients without RPR (0.435 versus 0.343 for AA-RPR and 0.484 versus 0.323 for PFA100/EPI-RPR).

At the multiple logistic regression analysis, the C807T/G873A GpIa gene polymorphisms resulted an independent risk factor for AA-RPR and for PFA100/EPI-RPR (Table 5). As concerns the GpIa polymorphism haplotypes, at the multiple analysis, TTA haplotype resulted an independent risk factor for PFA100/EPI-RPR (Table 5).

4. Discussion

In this study, we investigated the role of the C807T, G873A and T837C GpIa gene polymorphisms on modulating platelet function in patients with MI undergoing PCI on dual antiplatelet treatment. This study shows for the first time that the 807T/873A allele of the C807T/G873A GpIa gene polymorphisms is an independent risk factor for the RPR on antiplatelet treatment, and extends, in a larger acute coronary syndrome population, the observation that the 807T/873A allele is associated with higher platelet reactivity. Moreover, the evaluation of the three haplotypes obtained from the three polymorphisms also shows that the 807T/837T/873A haplotype is an independent risk factor for RPR on antiplatelet treatment.

Data from the present study confirm the complete linkage disequilibrium between 807C/873G and 807T/873A allele in agreement with previous reports [25,26]. The prevalence of the 807TT/873AA genotype (11.4%) was consistent with that reported in another Italian acute coronary syndrome population (12.7%) [27], similar to those reported for Spanish coronary syndrome populations (from 10 to 15.5%) [22,23] and lower than that reported for Greek MI population (20.9%) [28]. Differences could be due to the low number of patients evaluated in the previous studies. Concerning the T837C GpIa gene polymorphism, this is the first report of its prevalence in an Italian acute coronary syndrome population (prevalence of the 837TC + 837CC genotypes = 21.5%). This finding is consistent with previous data about a low prevalence of this polymorphism [21].

In addition to the evaluation of the stimulated platelet reactivity by PRP aggregation, we decided to evaluate the platelet function by PFA100, which explores adhesion-aggregation reactions in the presence of the other blood cells in condition similar to those in the circulation where platelets are exposed to high shear stress and agonists. Therefore, the evaluation of platelet function by PFA100 could permit us to study the role of GpIa gene polymorphisms in affecting the function of the GpIa/IIa receptor in the early steps of platelet adhesion to collagen.

Our data show that the presence of the C807T/G873A polymorphisms, but not the T837C polymorphism, is associated with higher platelet reactivity evaluated by both PRP aggregation and PFA100. Carriers of the 807T/873A allele had significantly higher platelet aggregation values after AA and collagen stimuli and, even if they did not reach the statistical significance, after ADP stimulus. Carriers of the 807T/873A allele had also significantly shorter CT/EPI values by PFA100, so supporting the effect of this allele in modulating also the early steps of platelet adhesion to collagen.

Table 5
Odds ratios for AA-RPR and PFA100/EPI-RPR according to C807T/G873A GpIa polymorphisms and C807T, T837C and G873A GpIa haplotypes

	AA-RPR		PFA100/EPI-RPR	
	Multiple analysis[a] OR (95% CI)	p	Multiple analysis[a] OR (95% CI)	p
C807T/G873A GpIa				
CC/GG	1		1	
CT/GA	1.1 (0.55–2.18)	0.800	2.8 (1.35–5.62)	0.005
TT/AA	3.0 (1.17–7.89)	0.022	4.1 (1.53–10.89)	0.005
GpIa Haplotypes				
CTG	1		1	
CCG	0.9 (0.38–2.05)	0.779	1.0 (0.43–2.18)	0.927
TTA	1.5 (0.92–2.42)	0.106	2.1 (1.29–3.35)	0.003

[a] Adjusted for age, gender, hypertension, diabetes mellitus, hypercholesterolemia, obesity, and smoking habit.

In a previous study on patients undergoing PCI on dual antiplatelet treatment at 24 h after the procedure statistically significant differences in platelet aggregation between carriers and noncarriers of 807T allele upon collagen, but not ADP, stimuli was observed [22]. In a further study on CAD subjects on dual antiplatelet treatment for >1 month, an increased platelet aggregation after ADP, collagen and epinephrine stimuli in 807T allele carriers compared with noncarriers was found [23]. Differences in ADP induced platelet aggregation could be due to differences in ADP concentrations used for platelet aggregation induction (2 μM and 10 μM ADP present study, 6 μM ADP [22], and 20 μM ADP [23]) and/or to the different length of dual antiplatelet treatment (24 h or >1 month). Therefore, the present findings show that the presence of the 807T/873A allele affects not only the specific collagen induced platelet aggregation but also platelet aggregation induced by other nonspecific stimuli, such as AA or, even if in a mild manner, ADP. Previous functional studies demonstrated that the two linked silent GpIa dimorphisms, C807T and G873A [20] or the three haplotypes obtained by analyzing C807T, T837C and G873A polymorphisms [21] were correlated with a variable expression of the platelet surface $\alpha_2\beta_1$ receptor, and that differences in the receptor density directly correlate with the rate of platelet adhesion to collagen under flow conditions [21]. Our data contribute to demonstrate that the increased expression of $\alpha_2\beta_1$ receptors, due to the presence of 807T/873A or 807T/837T/873A genetic profiles, determines a higher platelet reactivity independently from the applied stimuli.

At the best of our knowledge, our data show, for the first time, that the prevalence of the 807TT/873AA GpIa genotype is significantly higher in patients with RPR than in patients without RPR defined according to both AA-induced platelet aggregation (AA-RPR) and CT/EPI values (PFA100/EPI-RPR (20.3% versus 9.1% and 18.3% versus 9.2%). Moreover, we have demonstrated that the 807T/873A GpIa allele is a significant predictor of both the AA-RPR and PFA100/EPI-RPR (both phenomena aspects of the so called ASA resistance) in patients with MI.

The group of patients with PFA100/EPI-RPR is larger ($n = 71$) than that with AA-RPR ($n = 59$). Interestingly, among the 25 patients with PFA100/EPI-RPR only, 19 (76%) were heterozygous for the C807T/G873A polymorphisms (data not shown). This phenomenon could be due to the fact that PFA100 system is able to better explore the early steps of platelet adhesion and aggregation to collagen and then it is more sensible to the effect of increased number of GpIa/IIa receptors in subjects carrying the 807T/873A GpIa allele.

Upon vessel injury, adhesion proteins, including collagen, become exposed to circulating blood platelets. Platelets adhere to collagen via the GpIa/IIa receptor which is believed to play a major role in this process [14,16,29]. GpIaIIa receptor density on the platelet surface is controlled by genetic factors, including the C807T polymorphism in the GpIa gene [21,30]. Our findings improve the knowledge on the functional role of the C807T/G873A GpIa polymorphisms described in previous reports [20–23] and provide a possible explanation on the increased risk of MI and stroke previously observed in 807T/873A allele carriers [26,28,31,32]. Moreover, our data suggest that the C807T/G873A GpIa polymorphisms are involved in modulating individual response to dual antiplatelet treatment and in particular to ASA treatment. Recently, our group provided the first evidence that RPR, measured after primary PCI in patients with MI, is an independent predictor of major adverse coronary events after 1-year follow-up [11].

A limit of our study is the impossibility to finely discriminate the role of the GpIa gene polymorphisms in affecting platelet function in response to the two different treatments (ASA and clopidogrel) and to evaluate changes in platelet functional reactivity from the pre-treatment condition in the single patient.

These results provide meaningful indication on the role of C807T/G873A GpIa polymorphisms in the real world practice of dual inhibition in acute coronary syndrome patients, which consider mandatory this kind of antiplatelet strategy. Due to the fundamental clinical implications, further studies on larger populations with an adequate follow-up are needed in order to confirm the role of C807T/G873A GpIa gene polymorphisms and other polymorphisms in the same gene or other candidate genes as predictor of RPR and consequent complications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online Version, at doi:10.1016/j.atherosclerosis.2006.11.009.

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